

# Hypoxia, perfusion, and vascular leakage measurement

YS Yi Sun LC Lieping Chen YZ Yuwen Zhu

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Blockade of the CD93 pathway normalizes tumor vasculature to facilitate drug delivery and immunotherapy

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## Detailed protocol

Murine tumor Hypoxia, perfusion, and vascular leakage measurement protocol

### 1. Tumor bearing mice treatments

Tumor hypoxia was detected after injecting 30mg/kg pimonidazole hydrochloride (Hypoxyprobe kit) into tumor-bearing mice 1 hour before tumors were harvested. To detect the formation of pimonidazole adducts, tumor frozen sections were stained with APC-Hypoxyprobe mAb (1:100 dilution).

Tumor perfusion was detected by intravenous injection of 50ug FITC-labeled Lycopersicon esculentum (Tomato) lectin (FL-1171, Vector laboratories, Brussels, Belgium) into tumor-bearing mice 10 min before tumors were harvested for analysis.

Tumor vascular leakage was evaluated by intravenous injection of 5mg FITC-labeled Dextran (40KD, FD40S Sigma) into tumor-bearing mice 30 min before tumors were harvested for analysis.

### 2. Tumor cryosections

After tumor was harvested and frozen in OCT block, cryosections were made for downstream IF staining.

Cryosection thickness

Tumor hypoxia	8um
Tumor perfusion	8um
Tumor vascular leakage	30um

### 3. IF staining (Tumor perfusion and tumor vascular leakage)

- Acetone fixation -20°C 30min
- Wash 5min twice
- Blocking (2.5% Rat serum in PBS) 30min RT
- anti-mCD31-PE (Biolegend clone 390) 1:100 1h RT
- Wash 5min twice
- DAPI staining
- Wash 5min

### 4. Image quantification

- 10 random images were taken from each tumor tissue using Nikon Eclipse TE2000-E upright microscope.
- Data analysis was done by SlideBook software (Version 6, Intelligent Imaging Inc). The threshold to define positive area was determined by software automatically. The threshold value might be slightly different among individual experiment.
- The perfused tumor vasculature quantification was calculated as FITC+ CD31+ vessels / total CD31+ vessels.
- The vascular leakage quantification was calculated as FITC+ leaked area / tumor area.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sun, Y. , Chen, L. and Zhu, Y. (2021). Hypoxia, perfusion, and vascular leakage measurement. Bio-protocol Preprint. [bio-protocol.org/prep1487](https://www.bio-protocol.org/prep1487).
2. Sun, Y., Chen, W., Torphy, R. J., Yao, S., Zhu, G., Lin, R., Lugano, R., Miller, E. N., Fujiwara, Y., Bian, L., Zheng, L., Anand, S., Gao, F., Zhang, W., Ferrara, S. E., Goodspeed, A. E., Dimberg, A., Wang, X., Edil, B. H., Barnett, C. C., Schulick, R. D., Chen, L. and Zhu, Y. (2021). Blockade of the CD93 pathway normalizes tumor vasculature to facilitate drug delivery and immunotherapy. Science Translational Medicine 13(604). DOI: [10.1126/scitranslmed.abc8922](https://doi.org/10.1126/scitranslmed.abc8922)

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